Apoptosis research enters the ICE age

Recent elucidation of the structure of interleukin-1β-converting enzyme (ICE), a protease with sequence homology to a nematode protein associated with programmed cell death, opens a new chapter in the study of how proteases may control cellular suicide.

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Recently, the three-dimensional structure of interleukin-1β-converting enzyme (ICE), the protease responsible for cleaving IL-1β from its precursor to mature form, was solved by two independent groups [1,2]. While this has drawn significant attention from molecular immunologists and structural biologists alike, the information has also attracted the notice of scientists studying the process of apoptosis (also known as physiological or programmed cell death) [3]. While ICE plays a crucial role in initiation of the T-cell response by the immune system, it may also have a more fundamental role that strikes at the very heart of how every cell is able to survive. ICE shares 28% sequence identity with the CED-3 protease, the product of cell death gene-3 from the nematode *Caenorhabditis elegans* [4], a protein known to cause apoptosis. This connection between ICE and CED-3 has sparked intense interest in these proteases, suggesting that ICE or an ICE-like homolog may be participating in the complex cascade that will destine a cell for suicide by apoptosis. This article presents the molecular players in the field of apoptosis and gives a brief overview of the current state of research and future directions.

To live or to die?

Apoptosis is a controlled process by which an individual cell within an organism is eliminated. (For a recent review, see [5].) In multicellular organisms, homeostasis is achieved through a continual balance between cell proliferation, quiescence, differentiation and death, with death occurring by one of two general pathways: necrosis or apoptosis. Apoptosis is an active process that allows cell suicide to occur in an orderly manner without giving rise to the inflammatory response which is normally characteristic of cell death by necrosis. The apoptotic process is closely tied to cell proliferation whereby some of the same molecules that regulate progression through the cell cycle, such as the proteins myc and p53, can also serve at checkpoints to regulate whether a cell should initiate apoptosis [6–8]. The link between the processes of life (proliferation) and death (apoptosis) results from self-regulation, perhaps based on the ratio of proliferative and suicidal signals that are constantly being sensed by the nucleus of the cell. When signalling between these divergent life and death pathways loses its synchrony, as is the case in cells that are severely damaged by radiation or chemotherapeutic agents, the cell may respond with signals which activate apoptosis. The ability of an individual cell to recognize that it is too extensively damaged to be properly repaired would insure that mutated cells could be eliminated for the overall good of the organism. This may be an important regulatory mechanism for maintenance of homeostasis. Left uncontrolled, mutations which disrupt the balance between life and death signals could cause cells to proceed through uncontrolled proliferation [9]. While loss of proliferative control is a common feature of cancers, an unregulated propensity for apoptosis may lead to degenerative diseases, such as Alzheimer's disease [10,11] or AIDS [12].

Recent advances in cell and molecular biology have modified our views about how both normal and transformed cells live and die. Normal cells are capable of proliferating for a finite number of cell divisions. In response to cellular signalling (both from internal and external stimuli) an individual cell can either become terminally differentiated, become quiescent while awaiting an external stimulus, or undergo physiological cell death. It is generally believed that cancer cells possess a growth advantage that permits them to continue proliferating without reaching these finite limits. Moreover, it is becoming clear that some cancer cells also have a 'death advantage': some tumor cells lose the ability to die by apoptosis. In solid tumors, cells that escape growth control and lose responsiveness to signals that would trigger apoptosis contribute to the net accumulation (i.e. growth) of solid tumor mass.

Molecular basis of apoptosis

While the cellular mechanisms that control apoptosis are not fully understood, several important genes and their protein products that are capable of participating in initiation and progression of the cell death process have been identified and cloned (Fig. 1). Much of this information is based on fundamental research with *C. elegans*. The fate of each of the 1090 cells in *C. elegans* has been mapped during maturation [13]. Of these, 131 cells are destined to undergo apoptosis during development. From studies of these cells and the genes that control development, Ellis and Horvitz identified *ced-3* as critical for the process of apoptosis [14]. When this gene was mutated, cells that should have been eliminated during development remained viable. Studies of *C. elegans* and its cell death genes have continued for several years; however, it was not until 1993 that the relationship between

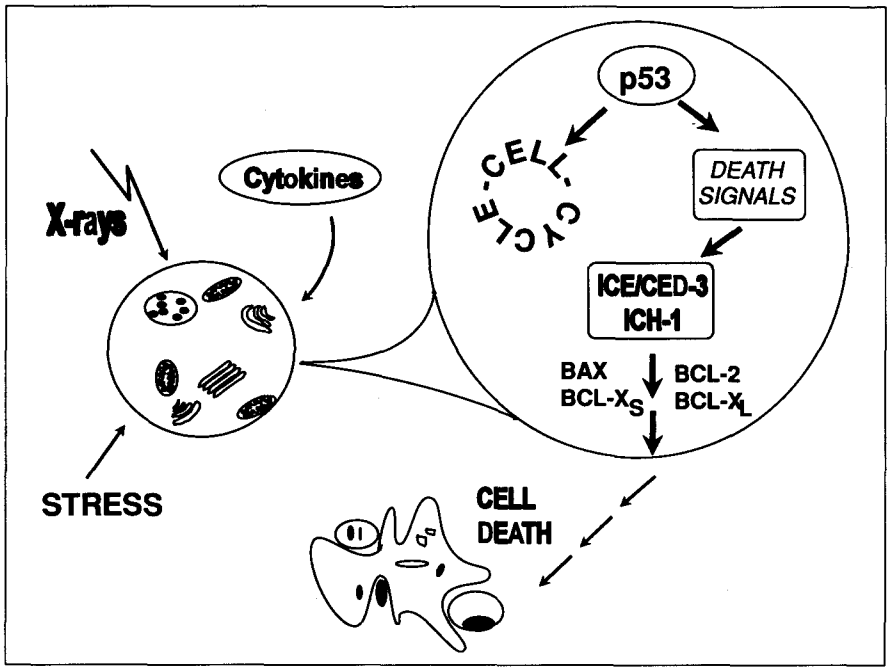

Fig. 1. A model for cell death by apoptosis. Apoptosis can be triggered in cells by a variety of factors, including genotoxic stress, damage by X-rays, activation by selected cytokines, or removal of growth factors. The tumor suppressor protein, p53, which also controls progression through the cell cycle, is thought to control the decision to undergo apoptosis. Cell death modulators, such as Bax/Bcl-X_S and Bcl-2/Bcl-X_L, act to promote or prevent cell death, respectively. An ICE-related protease may be responsible for activation or propagation of the death signal. The overall process of cell death involves overlapping pathways that ultimately result in chromatin condensation, nuclear disintegration, membrane blebbing, loss of cell volume and fragmentation of the cell into apoptotic bodies.

the CED-3 protease and the cellular protease ICE was recognized [4]. While the role of ICE itself as a component of the cell death machinery remains controversial, homology between these two proteins points to a commonality of function. Considerable effort has gone into demonstrating that ICE, like CED-3, is capable of causing apoptosis [4]. Several other proteins having partial sequence homology to ICE have been identified and characterized in the past year [15,16]. Together, these proteases represent a family of proteins that may be responsible for a wide variety of both positive and negative effects on apoptosis.

ICE and its homologs

ICE is a novel cysteine protease that is encoded as an inactive 45 kDa precursor protein [17]. Through autoactivation, an inactive dimer is cleaved to an active heterotetramer consisting of two p20 and two p10 subunits. An active-site cysteine residue (Cys285) within the sequence QACRG is conserved throughout this family of proteases which cleave target proteins selectively at aspartyl residues [17]. Important information about the catalytic mechanism and organization of subunits can be derived from the X-ray crystal structure of the protein [1,2]. The mechanism of peptide cleavage involves formation of a tetrahedral intermediate with histidine (His237), glutamine (Gln283) and arginine (Arg179) residues providing essential contacts required for substrate binding and stabilization of the intermediate complex [1,2]. The organization of the heterotetramer into an inter-digitated complex is unique among proteases, and represents a novel quaternary structure. It is thought that the active site is composed of a p20 and p10 subunit contributed by each precursor, rather than from the same precursor molecule. This type of organization may have implications for the control of ICE activation, although additional studies will be required to clarify the role.

The most recently identified ICE homolog, Ich-1 (ICE and CED-3 homolog-1), has been shown to exist in two forms, Ich-1_L (long) and Ich-1_S (short), as a result of alternative splicing of its messenger RNA [16]. These two forms have different effects on cell survival when transfected into cells. Whereas the long form promotes apoptosis, the short form acts as a dominant-negative repressor of cell death. As with the ICE heterodimer, it is thought that Ich-1_S binds to Ich-1_L, although direct interaction has not been demonstrated. The ability of Ich-1_L to promote apoptosis provides additional evidence for involvement of an ICE-like protease in controlling the onset of apoptosis [16].

Death by proteolysis?

Major questions remain unanswered about the role of ICE or an ICE-like homolog in initiation and/or progression of apoptosis. First, is ICE (or a homolog) responsible for direct activation of specific cellular enzymes that subsequently cause the DNA cleavage, membrane blebbing and/or volume changes often associated with apoptosis? Alternatively, is apoptosis caused by cleavage of other regulatory protein(s) that, in turn, activate the enzymes required for progression of these self-destructive cascades? These questions are worthy of consideration and even speculation, since they can lead to experimental hypotheses that can be tested, and may provide further insight into these processes.

Recently, an ICE homolog now known as prICE was shown to cleave poly(ADP-ribose) polymerase into an active enzyme that causes oligonucleosomal laddering and apoptosis-like morphology in a cell-free extract [15]. ICE is unable to substitute for prICE in this system, although the two proteases share target substrate sequence homologies. These data provide the first direct evidence for involvement of a protease having a similar

target sequence to ICE, although maybe not ICE itself, in apoptotic processes.

If ICE, or a homolog, acts alone as the direct activator of enzymes that participate in apoptosis, it seems necessary to invoke the existence of a set of apoptotic enzymes that have at least two common features: these target enzymes must exist in precursor forms that are inactive until cleaved, and these precursor proteins must share a common protease recognition site. Although possible, enzymes having diverse enzymatic activities while retaining these common structural features are unlikely to have survived evolution from the simplest multicellular organisms to higher creatures. Alternatively, if ICE were modulating apoptosis through activation of other regulatory enzymes, the cell death process could be further amplified. This latter situation seems more plausible. Together, ICE and its activated substrate(s) could be responsible for triggering numerous enzymatic cascades that lead to cell death. This would place an ICE-like protease further upstream in the process, giving it a more crucial role in the initiation process. What remains unexplained by our knowledge thus far, is what constitutes the ultimate monitor that decides whether the cell should live or die. If an ICE-like protease is the enzyme that triggers the process of apoptosis, what controls the protease?

Numerous regulatory mechanisms have evolved for controlling cellular processes. Often, these controls involve chemical modification of pre-existing molecules within a cell. This permits cellular control to be exerted in the absence of *de novo* protein synthesis. Whereas proteolytic cleavage of an inactive precursor would be an irreversible event, phosphorylation represents perhaps the most common mechanism for rapid and reversible modulation of cellular activities. Indeed, protein phosphorylation is an essential component of many regulated cellular pathways, including receptor-mediated signal transduction and cell proliferation. Given the wide variety of kinases available within a cell, and the requirement for activation of multiple enzymatic cascades during the program of apoptosis, perhaps a combination of proteolytic events and chemical modifications would propel the cell into apoptosis. Phosphorylation is known to play a significant role in controlling cell cycle progression [18,19]. A control mechanism for apoptosis involving phosphorylation might be expected, given the numerous parallel checkpoints shared by apoptosis and the cell cycle.

Future directions

As noted at the outset, much remains to be discovered before we will fully appreciate the processes that regulate a cell's decision to live or to die. Understanding the role of an ICE-like protease in the activation of its substrates may provide further insights into the steps downstream of this enzyme. Identification of factors that control ICE activation may provide a view of the upstream parts of this cascade. Clearly, the results of the last few years of research in this area indicate that an ICE-like protease sits at a pivotal point in the cascade leading to the cell death process. Given the rapid progress that has been made in this field, we certainly have entered a period that will be known as 'the ICE age' in apoptosis research. It is only a matter of time before we realize the full significance of the role that this enzyme and its homologs play in cell survival and cell death.

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